# CORRELATION OF LYSOSOMAL ACTIVITY AND INGESTION BY THE MANTLE EPITHELIUM[1,2]

GERRIT BEVELANDER AND HIROSHI NAKAHARA

*Department of Histology, The University of Texas Dental Branch, Houston, Texas 77025, and The Bermuda Biological Station for Research*

Despite the fact that the structure and function of the molluscan mantle has occupied the attention of investigators for well over one hundred years the role of the outer mantle epithelium in the elaboration of the shell is as yet poorly understood. On the basis of histochemical and radioautographic studies (Bevelander and Benzer, 1948; Bevelander, 1952), it was shown that both mucus secreted by the mantle and $Ca^{45}$ derived from the sea water environment were incorporated in the shell. Several other authors (Ojima, 1952; Wilbur, 1960; Kado, 1960; Tsujii, 1960) have also remarked on the fact that mucus and other formed substances play a significant role in shell formation. It was further shown by Nakahara (1962a) that $Ca^{45}$ injected into the adductor muscle was incorporated into the mantle mucus which later became an integral part of the calcified nacre.

The fine structure of the mantle of *Fabulina* was described by Kawaguti and Ikemoto (1962). These authors contend that the outer surface of the mantle is made up of three cell types. Mention is made of the presence of microvilli, mitochondria, Golgi bodies, endoplasmic reticulum, but the presence of lysosomes is not mentioned. Furthermore no specific function of the various cell types described was suggested.

The above references, although by no means complete as to the number of published studies dealing with the structural and functional aspects of the mantle epithelium, do, however, indicate that the chief interest and concern of these investigations was the identification of various substances elaborated and secreted by the epithelium and their subsequent identification in the formed shell.

It was previously shown by Nakahara (1962) that the outer surface of the mantle of *Pinctada martensii* ingests carmine particles placed in the extrapallial fluid. In an attempt to clarify and amplify the above observations we have examined this problem in more detail, utilizing electron microscopy, histochemistry as well as experimental physiological procedures.

Briefly stated, this report deals with a description of the results obtained following the procedures mentioned above, by means of which we have demonstrated differential absorption by the outer surface epithelium of the mantle. A description of the intracellular localization of acid phosphatase, lipids and mucopolysaccharides at the light level and the detailed structure of the ingesting cell follows. Finally the locus of ingested material, together with other remarks concerning lysosomal activity in the mantle cells, is described.

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## Materials and Methods

This study was carried out on the calico clam, *Macrocallista maculata*. Specimens were collected in Bermuda waters and for purposes of histological and E.M. studies the mantle was removed and fixed in (1) a 1% solution of osmium tetroxide in a phosphate buffer at a pH of 7.4 for one hour at 25° C. (2) Other specimens were fixed in a 6% solution of glutaraldehyde buffered in phosphate at pH 7.4 for 4–6 hours at 25° C. Following this treatment the tissues were repeatedly washed in phosphate buffer and fixed in osmium tetroxide for one hour as described above. Following fixation, tissues were routinely dehydrated, embedded in Araldite and were then sectioned for E.M. observations (thin sections) and also 1–2 $\mu$ sections which were stained with a 40% alcoholic solution of 1:1000 toluidine blue for observations with the light microscope.

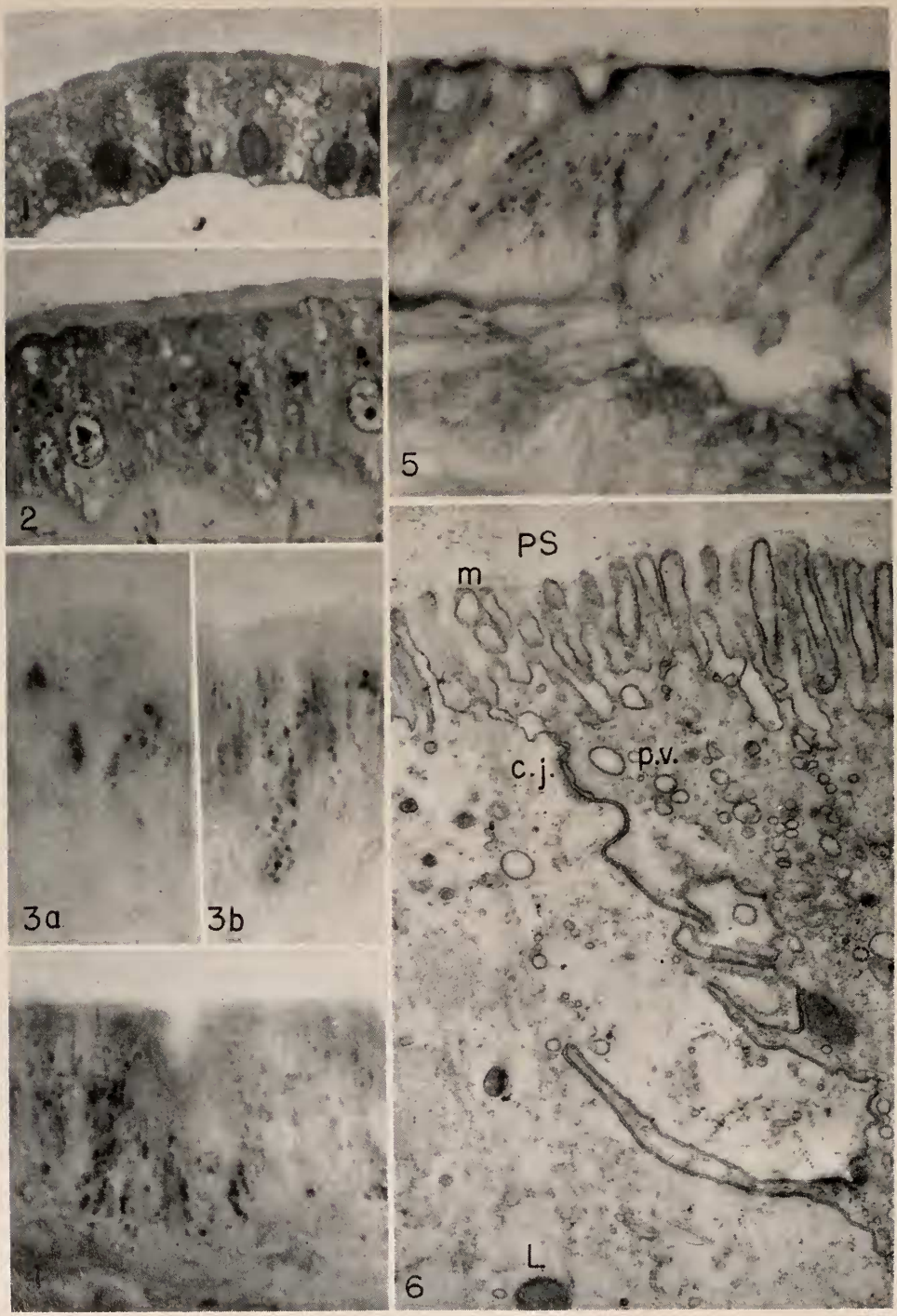
For the purposes of ascertaining the ability of the shell-forming epithelium to absorb particulate material the following procedures were carried out: 0.1 cc. of a 2% filtered sea water suspension of finely ground carmine particles was injected into the pallial space. Specimens were then placed in tanks furnished with running sea water at a temperature of 25–26° C. for a period of 1–3 days. At the termination of these periods they were removed from the tanks, the mantle was excised, fixed in Ca-formol, dehydrated and then sectioned for subsequent observation. This procedure was also carried out with several other specimens in which a sea water-colloidal gold (Hartman-Leddon) mixture was injected in place of the carmine. The colloidal gold mixture was prepared just prior to injection by adding equal amounts of aliquots of colloidal gold and sea water concentrated to 50% of their original volumes.

To aid in the identification of the ingestion sites, three histochemical procedures were utilized. They consisted in testing the mantle epithelium for the presence of acid phosphatase, according to the method of Gomori (1950), in the treatment of the mantle to ascertain the presence of lipids by the Sudan black B method (Chiffele and Putt, 1950), and the PAS method to demonstrate the presence of PAS-positive reaction sites.

## Observations and Results

When viewed by means of light microscopy, the cells of the outer fold appear typically columnar, containing basally located nuclei, folded cell membranes, numerous vacuoles and a prominent cuticular border (Fig. 1).

Examination of sections of the mantle epithelium following injection of carmine into the pallial space revealed uptake of dye particles throughout the entire surface of the outer epithelium of *Macrocallista*. The most pronounced uptake occurs in the epithelium of the outer surface of the outer fold, less pronounced in the thick region of the mantle distal to the base of the outer fold, while the remainder of the mantle epithelium (thin portion) exhibits still less dye uptake than the other regions. The localization of the dye is observed as granules of varying sizes, in some instances so small as to be hardly recognized by light microscopy; in others the granules are readily recognizable at a magnification of 400 × (compare Figures 1 and 2). In addition to the outer (shell) epithelium, numerous amoebocytes also exhibit marked uptake of the carmine. It should be noted that the distribution of




FIGURES 1–6.

the carmine particles observed in these cells varies considerably. One of the factors upon which this distribution is dependent is the time allowed for ingestion to occur.

Sections of the mantle examined following treatment for the demonstration of acid phosphatase show a localization of this enzyme in the epithelial cells to be similar in size and distribution to the localization of the carmine granules described above (Fig. 3a, b).

Examination of mantle epithelium stained with Sudan black B reveals the presence of lipids to be rather widely distributed, appearing as dark granules of approximately the same size and position as those observed in the epithelium exhibiting ingestion of carmine. Sections treated according to the PAS method and subsequently digested with saliva exhibit PAS-positive granules in the cytoplasm which correspond in size and location to granules observed by the other two methods mentioned above. Briefly, the localization of carmine, acid phosphatase, lipid and PAS-positive granules appears to be very similar (Figs. 4, 5). The presence of acid phosphatase, lipids and PAS-positive material in granules occurring in the cytoplasm is, according to Novikoff (1960), a criterion for the identification of lysosomes at the level of light microscopy.

In the paragraphs which follow we shall describe the structure of the epithelial cell as observed by the electron microscope. Although structural variations occur, the example we have chosen to illustrate exhibits most of the details which are characteristic of these cells. At the free surface one observes rather short microvilli which extend for a considerable distance into the underlying cytoplasm.

A prominent feature of these cells is the septate cell junction and the very pronounced folding of the cell membrane. Mitochondria are numerous and usually occupy a position in the distal half of the cell (Fig. 7). In several cells in this part of the mantle, the mitochondria appear to be undergoing degenerative changes, as shown by the poorly defined cristae and outer membranes. Ribosomes, cisternae and glycogen are much less prominent than in other regions of the mantle. The Golgi apparatus, not illustrated in our microphotograph, is also characteristic, usually appearing rather widely dispersed throughout the cell. The cells rest upon a well defined basement membrane and from this membrane scattered integumental fibers arise and extend to the free surface.

The presence of numerous micropynocytotic vesicles arising from the canaliculi

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Figures 1–5 are microphotographs of epithelium of the mid-portion of the outer mantle fold.

FIGURE 1. Typical columnar epithelium of outer mantle fold, showing cuticle (microvilli) on surface, folded cell membranes, numerous vacuoles and prominent basally located nuclei. Araldite section, stained with toluidine blue. × 1600.

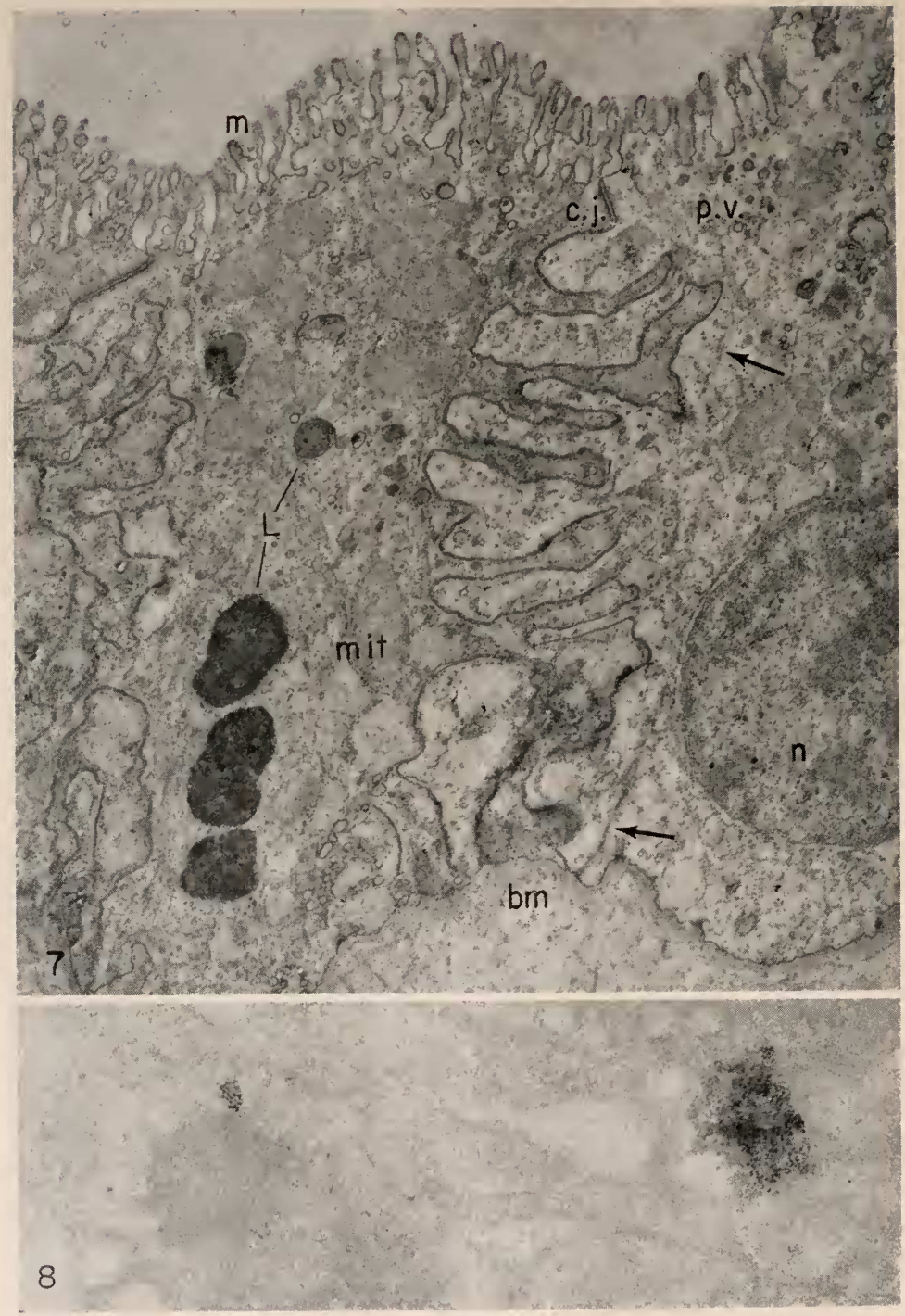
FIGURE 2. Section from specimen injected with carmine and fixed 24 hours later. Note distribution of fairly large dense granules indicating localization of carmine within the cell. Araldite section, unstained. × 1600.

FIGURE 3, a and b. Adjacent sections from specimen treated to show the localization of acid phosphatase indicated by dark granules. These photos illustrate the variation in enzymatic activity in adjacent parts of the mantle. × 1600.

FIGURE 4. Photograph of section fixed in Ca-formol and treated to show the localization of lipids indicated by the dark granules in the cytoplasm. × 1600.

FIGURE 5. This section was digested with saliva and then treated to demonstrate PAS-positive material. Note distribution of cytoplasmic granules. × 1600.

FIGURE 6. Electron micrograph of part of cell showing: c.j., cell junction; l, lysosome; m, microvilli; ps, pallial space; p.v., pinocytotic vesicles. Uranyl acetate stain. × 22,000.




FIGURES 7–8.

between the bases of the microvilli arranged in linear arrays is a characteristic morphological feature of these cells (Fig. 6). These arrays terminate in the proximity of larger vacuoles or lysosomes. The lysosomes vary in size, are enclosed by a single structural membrane and often contain electron-dense particles (Fig. 7). Examination of selected areas of cells injected with colloidal gold demonstrates as shown in Figure 8 that ingested particulate matter comes to be localized in cell organelles we have identified as lysosomes.

## Discussion

Our study of the mantle epithelium of *Macrocallista* confirms the previous observations of Nakahara (1962) for *Pinctada* that the epithelium associated with shell formation ingests particulate matter derived from the pallial fluid. He indicated that the ingested material came to be localized in the Golgi region. Our observations show that the Golgi apparatus in these cells is widely distributed and accordingly Nakahara's observations in this regard are essentially correct.

Novikoff (1960) has listed several examples illustrating pynocytosis as the mechanism responsible for ingestion and intracellular transport of substances which do not readily permit passage through the cell membrane. We have identified pynocytotic vesicles indicative of cytotic activity and in addition have utilized colloidal gold, recognized in the electron microscope, to trace the pathway of ingested particles from the cell surface to lysosomes located in various parts of the cell. In a similar study on the segregation of ferritin by glomerular epithelia, it was shown (Farquhar and Palade, 1959), that ferritin particles accumulate first in pynocytic vesicles, later in larger vesicles and finally in dense bodies or lysosomes. They assume that the same pathway is followed by other molecules, especially proteins of similar dimensions.

Our studies indicate that an apparently normal function of the mantle cells is the removal of particulate matter from the pallial fluid by means of pynocytotic activity. Studies currently in progress will attempt to show in more detail the nature of the materials removed by this method and also whether the removal of this material is associated with the mechanism of shell formation.

## Summary

1. Ingestion of particulate matter by the outer mantle fold of the calico clam, *Macrocallista maculata,* was studied. Following the introduction of carmine into the pallial space, dye particles were subsequently localized in the epithelia of the entire outer surface.

2. In an attempt to identify the cell structure in which the dye particles were localized, histochemical tests to identify acid phosphatase, lipids and mucopolysaccharides were employed. All of the above methods gave a positive reaction at the site corresponding to the locus in which the carmine was observed.

Figure 7. Electron micrograph of parts of two adjacent cells; uranyl acetate stain, × 13,000. bm, basement membrane; c.j., cell junction; l, lysosomes; m, microvilli; mit, mitochondria; n, nucleus; p.v., pinocytotic vesicles; tegmental fiber designated by arrows.

Figure 8. Electron micrograph showing ingested particles of colloidal gold localized in lysosomes; × 73,000.

3. Additional experiments were carried out in which colloidal gold was injected into the pallial fluid. Subsequent examination of epithelial cells showed that the colloidal gold was localized in organelles which, on the basis of fine-structure morphology and histochemical tests, we ascertain to be lysosomes.

4. Pinocytosis, occurring as a result of the pinching-off of the bases of the microvilli is a prominent activity of these cells. The micropinocytotic vesicles arising by this process apparently give rise to large vacuoles and lysosomes.

5. The intracellular mechanism by means of which ingestion by the mantle cells occurs has not previously been recorded. The significance of this activity awaits further study.

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